Molecular Characterization of a Brazilian Isolate of Bean Golden Mosaic Virus

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Bean golden mosaic was first reported in Brazil in 1961. It is now recognized as a serious constraint to bean production in Argentina, Brazil, Central America, and the Caribbean. This disease is caused by a bipartite geminivirus, bean golden mosaic virus (BGMV), which is transmitted by the whitefly, Bemisia tabaci. Previous research by R. Goodman and Associates provided sequences of the two ssDNAs (DNA A and DNA B) of an isolate (BGMV-PR) from the leguminous weed, Macroptilium lathyroides, collected in Puerto Rico (Howarth et al., 1985, PNAS USA 82:3572-3576). This isolate, which causes bean golden mosaic symptoms on Phaseolus vulgaris, has become the "standard" BGMV isolate and contrasts to the BGMV isolates from Brazil and Argentina by its mechanical transmissibility. Additional variability in BGMV is indicated by differences in the responses of the bean cultivars with moderate resistance to BGMV in different regions of Latin America. Thus, this project was undertaken to provide molecular characterization of the BGMV isolates and other bean geminiviruses found in Latin America. This information will be used in the development of DNA probes for the identification of specific BGMV isolates. These probes would allow the characterization of the genetic diversity of BGMV in a region, and this information would then be used in designing breeding strategies. Also, the sequence information is necessary for our future efforts to develop transgenic beans with resistance to BGMV

The BGMV isolate (BGMV-BZ) was collected in 1986 in Goiania Goias, Brazil from P. vulgaris with bean golden mosaic symptoms Several attempts to mechanically transmit this isolate were unsuccessful; however, this isolate was easily transmitted by whiteflies. DNA was extracted from infected plants in Brazil and sent to the University of Wisconsin. After agarose gel electrophoresis and ethidium bromide staining, DNA from BGMV-BZ-infected plants showed two predominant virus-specific DNA bands not present in DNA from healthy beans. These bands contained linear and circular dsDNA molecules (about 2.6 kb), and these circular molecules presumably corresponded to the dsDNA replicative form.

Partial- and full-length clones of DNA A and DNA B were prepared in suitable plasmids for sequencing by the dideoxynucleotide chain termination procedure. Both strands of full-length clones of DNA A and DNA B were completely sequenced as well as other independent clones which confirmed the DNA sequence through the full-length cloning site of HindIII and AccI for DNA A and DNA B, respectively. DNA A and DNA B were circular and were 2,617 and 2,580 bp in length, respectively, which is similar to the lengths for other characterized geminiviruses, eg, tomato golden mosaic virus (TGMV) and BGMV-PR.

Computer-assisted analysis of the sequences of DNA A and DNA B of BGMV-BZ was performed to determine the presence of open reading frames (ORFs) of at least 100 amino acids and to determine the sequence similarity of BGMV-BZ to other geminiviruses. DNA A has four ORFs and a region (the common region) of 181 nucleotides which is nearly identical to a similar region in DNA B. A common region has been identified in all bipartite geminiviruses and sequences in this region are nearly identical between the two DNAs, but differ among the various geminiviruses. Two ORFs were identified in DNA B. Thus, the genome organization of BGMV-BZ is similar to that of the other bicomponent geminiviruses (Lazarowitz, 1987, Pl. Mol. Biol. Reptr. 4:177-192).

Sequence comparisons were made among BGMV-BZ, BGMV-PR, and TGMV. Some regions showed clear similarities; however, there were many regions of considerable divergence. The nucleotide sequence homologies of the common regions between BGMV-BZ and BGMV-PR, and between BGMV-BZ and TGMV were 59% and 68%, respectively. A 15 nucleotide inverted repeat found in the common region of BGMV-PR was not present in the common region of BGMV-BZ, whereas a 14 nucleotide stem-loop region was present in the common regions of all three viruses. The sequence homologies for the ORFs in DNA A between BGMV-BZ and BGMV-PR and between BGMV-BZ and TGMV were similar and varied from about 72% for the putative polymerase gene to 82% for the putative coat protein gene. The comparisons of the sequence homologies of the ORFs in DNA B among the three geminiviruses were between 72% and 80%. The sequence divergence between BGMV-BZ and BGMV-PR was as great as the divergence between BGMV-BZ and TGMV. Thus, BGMV-BZ and BGMV-PR should be considered strains of BGMV.

The results of the sequence comparisons between BGMV-BZ and BGMV-PR indicate that it will be possible to identify suitable regions for use either as a universal probe to identify all BGMV isolates or as isolate-specific probes.

Future research will involve the molecular characterization of isolates of BGMV from Guatemala and the Dominican Republic. Since these isolates are mechanically transmissible, it is expected that the full-length clones will be infectious when applied to plants. These infectious clones then will be used in site directed mutagenesis studies to determine the functions of the ORFs as well as in the development of transgenic beans with resistance to BGMV